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Identification of genes that interact with *liquid facets*

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Identification of genes that interact with *liquid facets*

by

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Thesis

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Dedication

To myself, my friends, and family

Acknowledgements

I thank my advisor, Janice Fischer, for motivating me and tolerating me, and for allowing me to work in the lab. I also thank Juan Raudales, an undergraduate interested in biology who helped me perform experiments for one year in the lab. In addition, I thank members of the Fischer Lab who provided reagents and helped me learn experimental techniques. I also acknowledge my friends and family, who provided moral support through my time in graduate school.

Identification of genes that interact with *liquid facets*

by

Gerrit Alexander van der Ende, MA

The University of Texas at Austin, 2012

Supervisor: Janice Fischer

The protein Liquid facets (Lqf) promotes endocytosis at the plasma membrane[1]. Lqf activity is required for proper Notch signaling, likely through facilitating the endocytosis of Notch ligand by indirectly linking ligand to clathrin. A genetic modifier screen to identify genes that interact with *lqf* was performed by a previous graduate student. Genes identified in the screen might provide new insights into how Lqf promotes endocytosis or how Notch signaling is regulated. In this work, I performed genetic mapping techniques to identify the genes mutated in each complementation group of the screen. I identified the gene mutated in complementation group 6 as *mitochondrial alanyl tRNA synthetase* (*Aats-ala-m*). tRNA synthetases link a tRNA to its cognate amino acid during translation. Mitochondrial tRNA synthetases function in the mitochondria in translation. *Aats-ala-m* genetically interacts with *lqf*, suggesting the two genes function in the same pathway. In this work, I also identified chromosomal regions where the genes mutated in complementation groups 1,2, and 9 are located.

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Chapter 1: Introduction

Notch signaling in development

Notch signaling is a conserved pathway throughout the animal kingdom and functions in a diverse set of cellular contexts. Notch signaling plays a part in cell fate determination during organism development by mediating communication between adjacent cells. In Notch signaling, Notch receptor on the plasma membrane is activated through the interaction between the receptor and Notch ligand of the DSL family on the membrane of an adjacent cell. Differences in the strength of signal sending and receiving between the two cells lead to one cell adopting a signal sending fate and the adjacent cell having a signal receiving fate. The cells will then take separate lines of differentiation.

Mechanism of Notch signaling

Notch receptor at the plasma membrane is a heterodimer composed of an extracellular N-terminal fragment and transmembrane/intracellular C-terminal fragment[3]. Interaction with ligand on an adjacent cell leads to two cleavages of the Notch receptor. The second cleavage by the γ-secretase complex generates the Notch intracellular domain (NICD)[3]. NICD translocates to the nucleus where it promotes transcription of Notch target genes, in association with the DNA binding proteins Suppressor of Hairless Su(H) and Mastermind (Mam)[4].

Endocytosis of the ligand is required in Notch signal sending for activation of the Notch receptor. One model is that endocytosis of ligand generates a pulling force that exposes a protease cleavage site on the Notch receptor[3]. The *Drosophila* Epsin Lqf is involved in endocytosis of ligand. Fat facets (Faf) deubiqutinates Lqf, leading to stabilization of Lqf[5]. Endocytosis of ligand is promoted by the E3 ligases Neuralized and Mindbomb, which ubiquitinate ligand[6]. Endocytosis is also required in the signal receiving cell for proper Notch signaling. It is not known why endocytosis is required in the receiving cell[3].

Lqf in development

Lqf is required in Notch signaling in *Drosophila* in the signal sending cell. It is likely that Lqf is involved in all Notch signaling events throughout the organism[1]. At the N-terminus, Lqf has an Epsin-N-terminal homology (ENTH) domain which binds PIP_2 (phosphatidylinositol4,5-bisphosphate) at the plasma membrane[1]. Lqf has two Ubiquitin interaction motifs (UIMs) which are required for ubiquitination of Lqf and mediate the interaction between Lqf and ubiquitaned proteins. There are two Clathrin binding motifs (CBMs) on Lqf which allow Lqf to bind to clathrin. Also, Lqf has seven DPW motifs and two NPF motifs. The DPW motifs promote binding of Lqf to AP-2, and the NPF motifs promote binding to endocytic factors containing an EH (Eps15-homology) domain. A model for how Lqf promotes endocytosis of ligand is that Lqf associates with ubiquitinated ligand at the plasma membrane and associates with clathrin binding

endocytic factors such as the AP-2 complex, thereby indirectly linking ligand to clathrin[1]. Lqf is also involved in promoting curvature of the plasma membrane[7].

Lqf is involved in cellular events other than endocytosis and Notch signaling. The two yeast lqf homologs, ENT1 and ENT2 interact through the ENTH domain of these proteins with GTPase activating proteins (GAPs) of Cdc42, a regulator of the actin cytoskeleton[2]. In human cells, the Lqf homolog Epsin 1 associates with ubiquitinated EGF Receptor (EGFR) and promotes internalization of EGFR[8].

Endocytosis

Clathrin mediated endocytosis (CME) is a process that occurs at the plasma membrane where vesicles form that are coated with a complex set of proteins including clathrin, and are then internalized into the cytoplasm[9]. In CME, at the site of a forming clathrin coated vesicle, clathrin is linked to a receptor within the plasma membrane by an adaptor protein such as AP2. Dynamin at the neck of the forming vesicle pinches the vesicle from the plasma membrane[9]. Hsc70, with the assistance of Auxilin, uncoats the adaptor proteins and clathrn from the clathrin coated vesicle. The vesicle then fuses with an early endosome. Additional proteins such as Rab5, Avl, Hrs, and Rab11 mediate endosomal sorting. The contents of the vesicle traffic to the late endosome and eventually the lysosome or are recycled back to the plasma membrane.

Chapter 2: Results

A screen was performed by Ben Dolan to identify genes that interact with *lqf* (fig. 1). Bomsoo Cho performed complementation testing of the alleles isolated in the screen and found that the alleles group into 9 complementation groups, based on the observation that combinations of the alleles cause lethality. Bomsoo performed meiotic mapping of alleles from all complementation groups, and found that the *Neuralized* gene is mutated in group 3, *C-terminal Src Kinase* (*Csk*) is mutated in group 4, and *trio* is mutated in group 7. For several the remaining complementation groups, Bomsoo also found deficiencies that failed to complement the lethality of the alleles from the screen. I set out to identify the genes mutated in complementation groups 1,2,5,6,8, and 9.

Group 1

Meiotic mapping results suggested that the gene mutated in group 1 is located between the markers *stripe* (*sr*) and *ebony* (*e*) on the right arm of chromosome three. There are four group 1 alleles, *04, G7, S2*, and *J12*. I carried out deficiency mapping to identify the chromosomal region containing the mutant gene. If the mutant gene is within the breakpoints of a deficiency, it is expected that a fly carrying one third chromosome copy of the deficiency and one third chromosome with an allele of the mutant gene would not be viable, since a proper level of gene product is required for viability. I performed crosses between lines of flies with alleles of the gene to flies

containing molecularly mapped deficiencies which remove portions of DNA between *sr* and *e*, and analyzed phenotypes to determine if *deficiency/group 1 allele* flies were present in the progeny of the cross. I found that *bsc516* causes a lethality phenotype in combination with *04, G7, S2*, and *J12* (fig. 2). *Bsc141* causes a lethality phenotype with *G7* and *S3*. *bsc124* is viable in combination with all four alleles. From the mapping results, the Group 1 gene is likely between the 5' breakpoint of *bsc141*, and 5' breakpoint of *bsc124*, between 16,420,602 and 16,561,028. There are 13 genes in this region. *bsc141/O4* flies have an escaper phenotype, meaning that there is a reduced number of viable flies of this genotype in the F1 generation of the cross compared to the expected number of flies. It is not clear why *04* is non-viable in combination with *bsc516* but has an escaper phenotype in combination with *bsc141*. One explanation for why *04* is lethal with *bsc516* but not *bsc141* is that *bsc141* may partially delete the group 1 gene, or disturb a regulatory region of the gene, while *bsc516* completely deletes the gene. It is possible that the Group 1 gene is slightly upstream of the 5' breakpoint of *bsc141*. Alternatively, the group 1 gene may be within the region deleted by both *bsc516* and *bsc141*, and the *bsc516* deletion in combination with *O4*, like *bsc141*, causes only a reduction in viability, but extraneous factors such as the genetic background of one of the chromosomes enhances the reduced viability to lethality.

I next attempted to identify the Group 1 gene by complementation testing of alleles of genes in the region with the group 1 alleles. Alleles of the group 1 gene are

homozygous lethal, and lethal in transheterozygous combination. This suggests that reduced protein levels of the group 1 gene result in lethality. If an allele of a gene in the region identified by deficiency mapping results in reduced protein levels of the gene, and I find that the allele is lethal in transheterozygous combination with the group 1 alleles, this would suggest that this is the gene that is mutated in complementation group 1. I obtained alleles of 7 of the genes in the region that contains the mutant gene (table 1). To determine if the alleles result in reduced protein activity of each gene, I tested if each allele is lethal in transheterozygous combination with *bsc516*. *bsc516* completely overlaps all seven genes, so there should be no gene product from any of the seven genes from a *bsc516* chromosome. Assuming that the gene is required for viability, if *bsc516/allele* flies are non-viable, this would suggest that the allele of the gene results in a decrease in levels of the protein encoded by the gene below a level required for viability. I found that flies transheterozygous for *bsc516* and alleles of six of the genes are non-viable (table 1), suggesting that each allele causes reduced protein levels of the gene. I found that flies transheterozygous for each allele and alleles of the group 1 gene are viable. This suggests that the group 1 gene is not one of the genes tested by complementation testing. The group 1 gene is likely one of the six genes not tested by complementation testing, or a gene that is deleted in *bsc516*, but upstream of the 5' breakpoint of *bsc141*.

lqf^{FDD9} is a hypomorphic, temperature sensitive allele of *lqf*. *lqf*^{FDD9} homozygous

flies are viable and have phenotypes due to loss of Notch signaling in the eye, wing, and leg tarsal segment[10]. *lqf*^{FDD9} *J12/lqf*^{FDD9} flies are non-viable. This might mean that proper group 1 gene activity is important for lqf function.

Group 2

I set out to identify the gene mutated in complementation group 2. There are two group 2 alleles, *F1* and *S5*. Flies that are triply heterozygous for *faf*^{BX4}, *lqf*^{FDD9}, and *F1* or *S5* have rough eyes (fig. 3). Since there is a strong requirement for proper levels of all three proteins, this might mean that *lqf, faf,* and the group 2 gene are closely involved in a common biological process.

The gene mutated in group 2 was mapped between *hairy* (*h*) and *curled* (*cu*). Bomsoo Cho performed mapping with deficiencies and found that *Df(3R)Me15 fails* to complement the lethality of complementation group members *F1* and *S5*. I obtained deficiency mapping results which suggested that the mutant gene did not map to the region deleted by *Df(3R)Me15* (fig. 4) and found that the deficiency does not exist on the chromosome listed as *Df(3R)Me15* (fig 5). I then tested if the gene is located in other regions between *h* and *cu*. 61 molecularly mapped deletions in this region were tested to determine if they fail to complement the lethality of *F1* and *S5*. I found that *ed4685* fails to complement both complementation group members. I then found that *bsc414, bsc432*, and *ex6131*, which either partially or completely overlap *ed4685*, fail to

complement *F1* and *S5* (fig. 6). These results allowed me to narrow down the region containing the gene to 3L:17231345-17332971. There are nine genes within this region (fig. 7).

I sequenced the DNA of *cg7707, cg13723, cg6485*, and *cg6479* from the alleles *F1* and *S5* and did not find a mutation. This result suggests that the gene mutated in group 2 is not one of these genes. I performed complementation testing to determine if the group 2 gene is *cg6512*. The $cg6512^{EY04074}$ allele is a *P{EPgy2}* insertion in the *5' UTR* of *cg6512*. $cg6512^{EY04074}$ fails to complement the lethality of an overlapping deficiency. This suggests that the $cg6512^{EY04074}$ allele causes a decrease in protein activity of Cg6512. Flies that are transheterozygotes for $cg6512^{EY04074}$ and alleles of the group 2 gene are viable. This suggests that the group 2 gene is not cg6512.

Most likely, the group 2 gene is *cg6497, cg13724, cg13725* or *Odorant receptor 74a (*Or74a). *cg6497, cg13724*, and *cg13725* have no known conserved domains. *Or74a* is a member of a family of 60 odorant receptor genes in *Drosophila*[11]. Odorant receptors are 7 pass transmembrane G-Protein coupled receptors (GPCRs) that bind to odorant molecules and elicit a neuronal response. Except for *cg13724*, *cDNA* and *EST* evidence suggests that these are functional genes.

Alternatively, it is possible that the group 2 gene is not *cg6497, cg13724, cg13725*, or *Or74a*. The group 2 gene may be *cg6512*. The $cg6512^{EY04074}$ allele is lethal in combination with deficiency, presumably because Cg6512 protein levels fall below a

level required for viability. *F1* and *S5* may be alleles of *cg6512* that have some residual Cg6512 protein activity, and if $cg6512^{EY04074}$ has some residual protein activity, there may be enough protein activity for flies that are tranheterozygous for $cg6512^{EY0407412}$ and alleles *F1* and *S5* to be viable. *cg6512* encodes a mitochondria targeted protein. *cg6512* bares sequence similarity to human *AFG3-like protein 2*. AFG3-like protein 2 is part of a protease complex on the inner-membrane of the mitochondria[12]. It is also possible that the group 2 gene is located slightly upstream of the 5' breakpoint of *ex6131*. *ex6131/F1* or *S5* flies might be lethal because transcription of the group 2 gene is disrupted by *ex6131*. The 5' breakpoint of *ex6131* comes close to the transcription start site of *RNA binding protein 6* (*Rbp6*), a gene with large intronic sequences that encompasses 170kb. It is possible that *Rbp6* is the group 2 gene. A third possibility is that the group 2 gene is within the 3L:17231345-17332971 region, but it has not been identified as a gene.

Group 5

Complementation group 5 contains fourteen alleles. Bomsoo Cho performed meiotic mapping, and the results suggested that the mutant gene is located between the marker *ebony* and the telomere, on the right arm of the third chromosome. Bomsoo performed mapping with 22 Exelixis deficiencies, and found that *ex6210* is lethal or causes a reduced viability phenotype in combination with several of the Group 5 alleles.

I found that a deficiency which partially overlaps *ex6210, bsc567*, also is lethal or causes a reduced viability phenotype in combination with several of the Group 5 alleles. However, deficiencies overlapping *bsc567* and *ex6210* were viable in combination with group 5 alleles. This result suggested that the gene mutated in group 5 was somewhere else between *ebony* and the telomere. Juan Raudales and I performed mapping with deficiencies in regions telomeric to ebony that were not originally tested by Bomsoo (table 2). No deficiencies were found to be lethal with the complementation group 5 alleles. There are regions between *ebony* and the telomere that are not uncovered by deficiencies, and it is likely that the gene mutated in group 5 is in one of these regions.

Group 6

I also set off to identify the gene mutated in complementation group 6. Group 6 contains alleles *S1* and *B1,* which are homozygous lethal. The *S1/B1* transheterozygote is lethal, and heterozygous mutation of *S1* or *B1* enhances the lqf^{FDD9} eye phenotype (fig. 8). Bomsoo found that the group 6 gene maps between *ru* and *h* on the left arm of chromosome 3, and found that flies that are transheterozygous for deficiencies *ed210* and *ex6103* are non-viable in combination with the group 6 alleles (fig. 9) and flies transheterozygous for *ex6104* and the group 6 alleles are viable. I found that deficiencies *bsc557, bsc371*, and *bsc372* in combination with alleles *S1* and *B1* cause lethality (fig. 9). From this result, I narrowed down the region containing the mutation

to 3L:5129360..5177896. The *PBac{SAStopDsRed}* element is designed to generate loss of function mutations in genes where an insertion of the element occurs by splicing a stop codon into the open reading frame of the gene[13]. The *PBac{SAStopDsRed}LL05514* line has an insertion of the element in the third intron of the *Aats-ala-m gene*. I found that flies transheterozygous for *PBac{SAStopDsRed}LL05514* and *S1* or *B1* are non-viable. This suggests that *Aats-ala-m* is the gene mutated in group 6. *PBac{SAStopDsRed}LL05514* is also lethal with overlapping deficiencies. The identity of the complementation group 6 gene as *Aats-ala-m* was confirmed by my finding that expression of wild-type *Aats-ala-m cDNA* rescues the lethality seen in combinations of alleles *B1, S1*, and *PBac{SAStopDsRed}LL05514* (fig. 10). Sequencing of the mutant chromosomes showed that the *Aats-ala-m*^{S1} allele is a nonsense mutation that introduces a stop codon at amino acid 9 (fig. 11). The *Aats-ala-m*^{B1} allele is a missense mutation at amino acid 53 that changes a glycine to aspartic acid within the active site (fig. 12).

Phylogenetic analysis suggests that the gene I identified encodes the *Drosophila* mitochondrial alanyl tRNA synthetase. The Aats-ala-m sequence shows similarity to mitochondrial alanyl tRNA synthetases from other species[14]. Further support for the idea that the gene is a *mitochondrial tRNA synthetase* is that the protein encoded by *Aats-ala-m* aminoacylates mitochondrial-tRNA^{ala} in vitro[15].

Aminoacyl tRNA synthetases (Aats) link an amino acid to its cognate tRNA during protein translation. Most organisms have two nuclear encoded *Aats* genes for a given

amino acid. One Aats is involved in aminoacylation in the cytoplasm and the other aminoacylates in the mitochondria. Aminoacyl-tRNA synthetases often have cellular functions in addition to their role in aminoacylation. They are involved in a range of cellular processes including apoptosis[16], cytokine signaling[17], and translational silencing[18]. Mitochondrial aminoacyl-tRNA synthetases play a role in splicing of mitochondrial Group 1 and 2 introns[19].

Group 8

The gene mutated in group 8 was mapped meiotically between *ru* and *h*. Stephen Fleenor performed complementation testing using deficiencies between *ru* and *h*, and did not find any deficiencies that were lethal in combination with group 8 alleles *G6* and *K7* (table 3). These deficiencies did not uncover the entire region between *ru* and *h*. Juan Raudales performed complementation testing using deficiencies that uncovered regions not previously tested, and did not find any deficiencies that were lethal in combination with group 8 alleles. In addition, I performed complementation testing with deficiencies and did not find any that were lethal with group 8 alleles. There are regions of DNA between *ru* and *h* not uncovered by any deficiencies, and the group 8 allele may be located in one of these regions.

Group 9

The gene mutated in group 9 was mapped between *h* and *c*u. Bomsoo found deficiencies *81k19* and *ex6130* were lethal in combination with complementation group members *J12* and *05* (fig. 13). I performed additional complementation testing with deficiencies in the region. I found that *ed223* is lethal in combination with *05* and *J12*, *ed4674* is lethal in combination with *05* and *J12*, *ex9002* is lethal in combination with *J12* and viable with *O5*, *ed4606* is viable with *05* and *J12*, and *bsc561* and *ex9004* are viable with both *O5* and *J12*. The most likely explanation for these results is that the gene mutated in complementation group 9 is located in the region from the 3' end of *ed4606* to the 3' end of *ex6130*, between 16,773,223 and 16,799,748. *ex9002* may be lethal in combination with *J12* but not *O5* if *J12* is a stronger allele than *O5*, and the deletion disturbs a regulatory region of the group 9 gene and is a hypomorphic allele of the gene.

The complementation group 9 gene is likely one of the four genes located in the 16,773,223 and 16,799,748 region. *cg9674* encodes a protein with sequence homology to the *glutamate synthase* family of genes. Glutamate synthases are present in bacteria, plants, and insects and catalyze the synthesis of glutamate from glutamine and 2-oxoglutarate[20]. *Int6* is a subunit of Eukaryotic translation initiation factor 3 and also a regulator of the proteasome[21]. *cg9706* is the Drosophila member of the Acetyl-CoenzymeA transporter family[22]. Acetyl-CoenzymeA transporters are transmembrane proteins of the Endoplasmic Reticulum (ER) that transport acetyl Coenzyme A into the

lumen of the ER/Golgi Apparatus. Acetyl Coenzyme A is used in the Golgi for O-acetylation of proteins, a form of post-translational processing. *cg9705* has a nucleic acid binding domain (*flybase.org*).

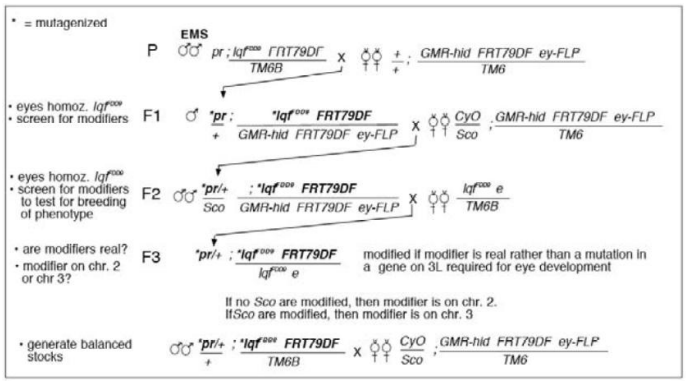

Figure 1. *lqf*^{FDD9} genetic modifier screen performed by Ben Dolan

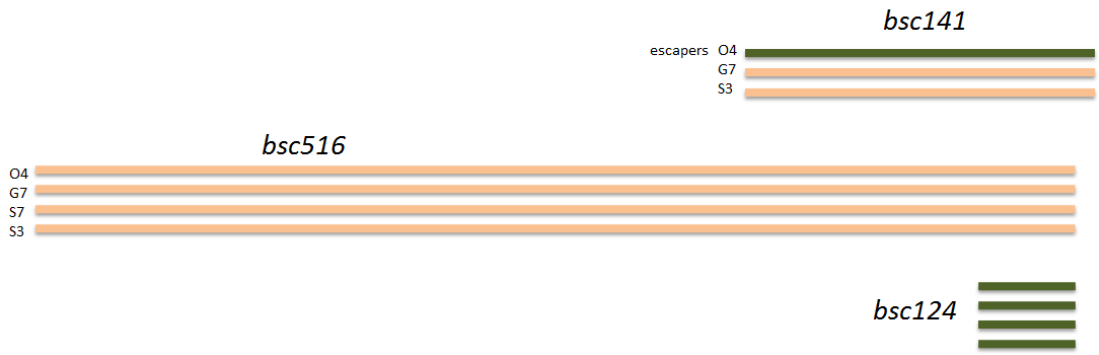

Figure 2. Group 1 deficiency mapping results.

The tan bars represent *deficiency/allele* combinations that are non-viable, and the green bars represent *deficiency/allele* combinations that are viable.

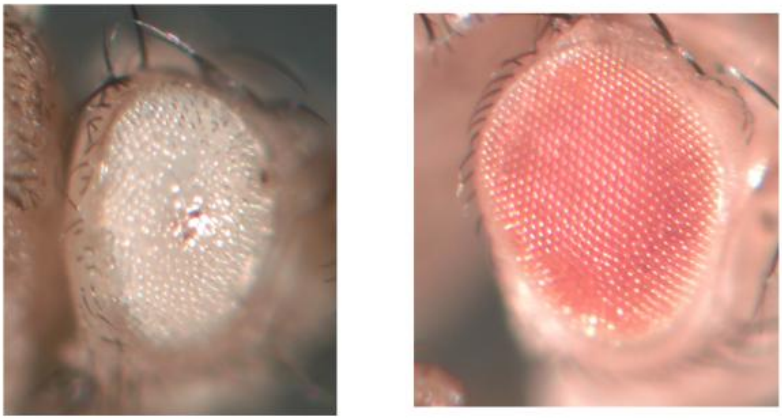

Figure 3. Triple heterozygotes display a rough eye phenotype

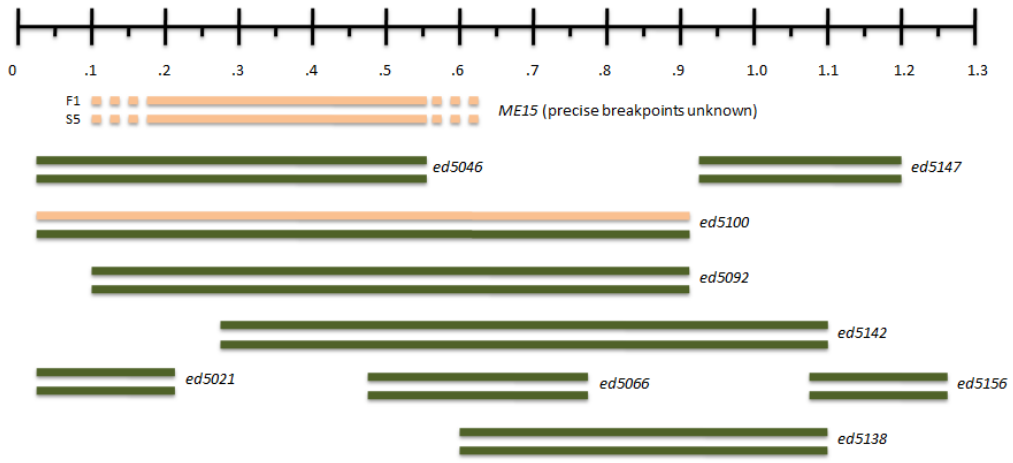

Figure 4. Deficiency mapping around the region deleted by *Df(3R)Me15.*

The exact sequence coordinates of *Df(3R)Me15* are not known. *Df(3R)Me15* was mapped to this region by complementation testing. Overlapping deficiencies generally complemented the lethality of *F1* and *S5*. This suggests that the gene mutated in complementation group 2 is located somewhere other than this region.

Df(3R)ME15 mapped by cytology to 81F3-82F7:
beginning of right arm of third chromosome

Previous complementation mapping[23,24,25]

	hkb- 170kb	*tacc*- 560kb	*hd*- 1,198kb
Df(3R)ME15	NV	NV	V

Complementation mapping performed by me

	tub- 212kb	*Karyβ3*- 474kb
Df(3R)ME15	V	V

Figure 5. Complementation testing suggests deficiency is not present

The genes *hkb, tacc*, and *hd* are required for viability. Previous groups showed that flies transheterozygous for alleles of *hkb* and *Df(3R)ME15,* and *tacc* and *Df(3R)ME15* are non-viable, suggesting the deficiency removes the chromosomal region containing *hkb* and *tacc*. Complementation testing that I performed suggests that the deficiency is not present on the *Df(3R)ME15* chromosome stocked in our lab. The genes listed are: *huckebein (hkb), transforming acidic coiled-coil protein (tacc), humpty dumpty (hd), tube (tub), Karyopherin β3 (Karyβ3).*

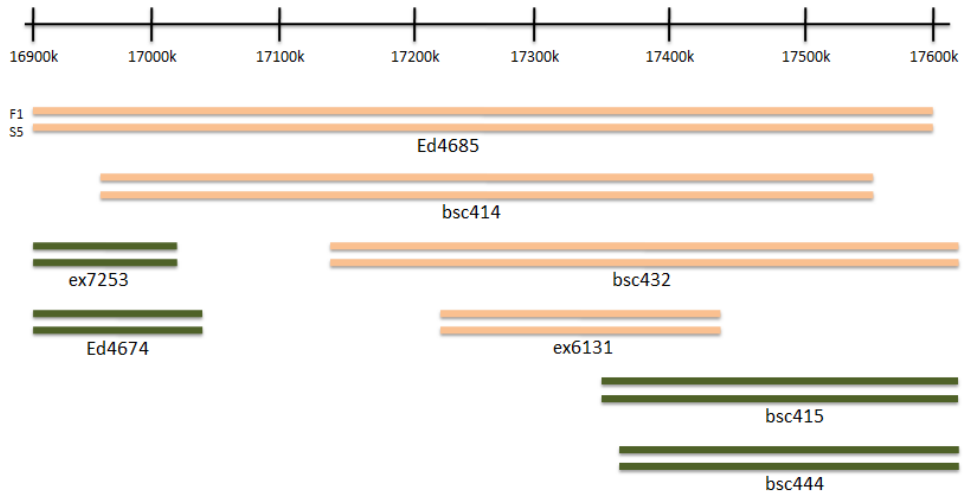

Figure 6. Group 2 Deficiency mapping results

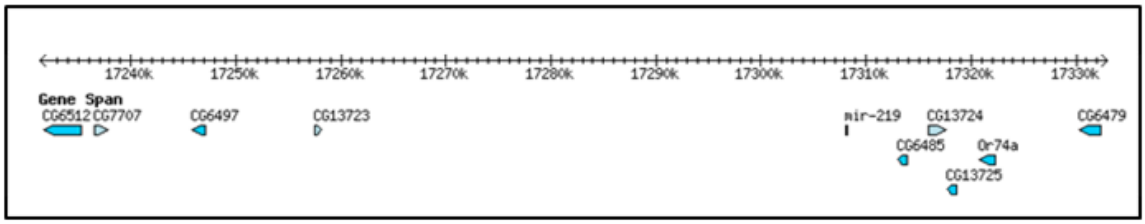

Figure 7. Region between 5' breakpoint of *ex6131* and 5' breakpoint of *bsc415*.

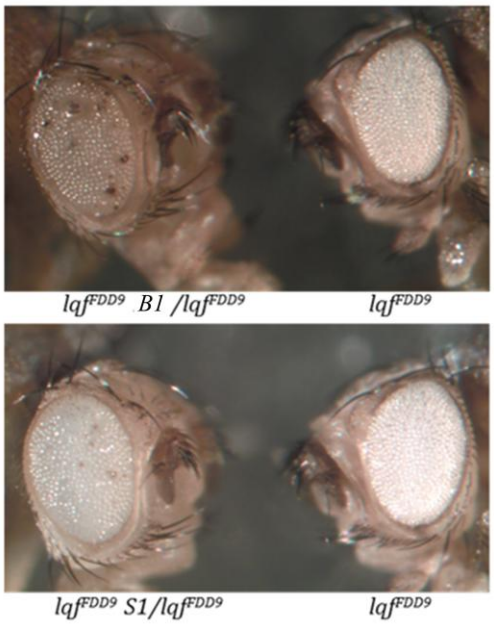

Figure 8. *B1* and *S1* alleles enhance the *lqf*^{FDD9} rough eye phenotype

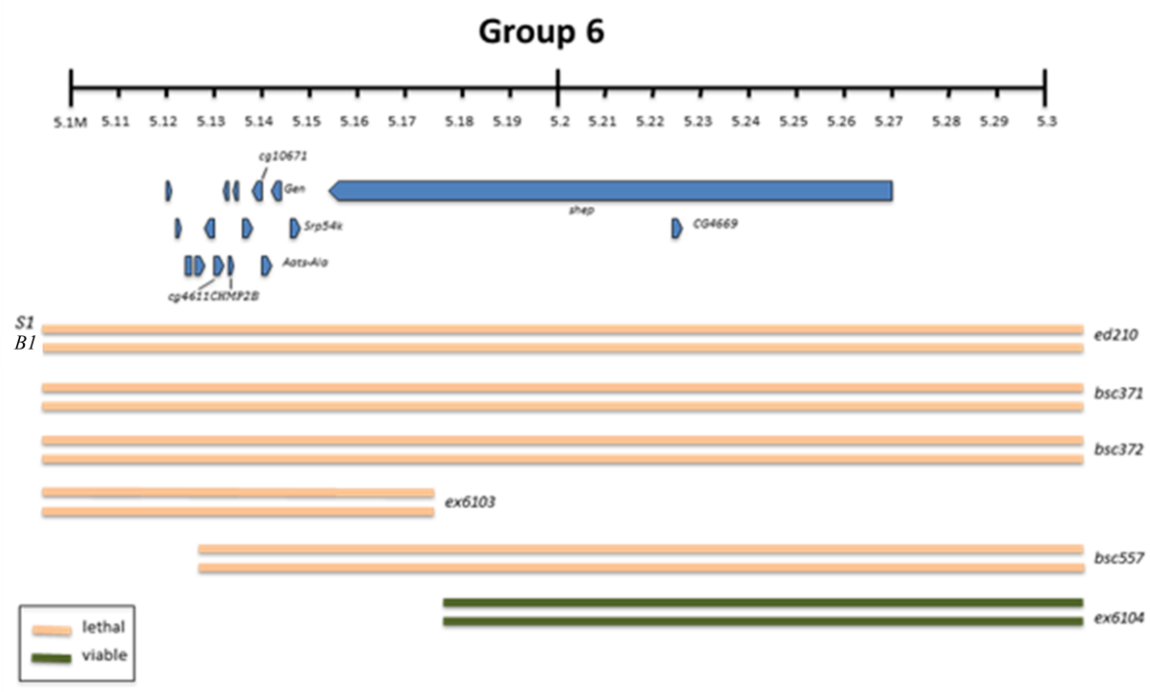

Figure 9. Group six deficiency mapping

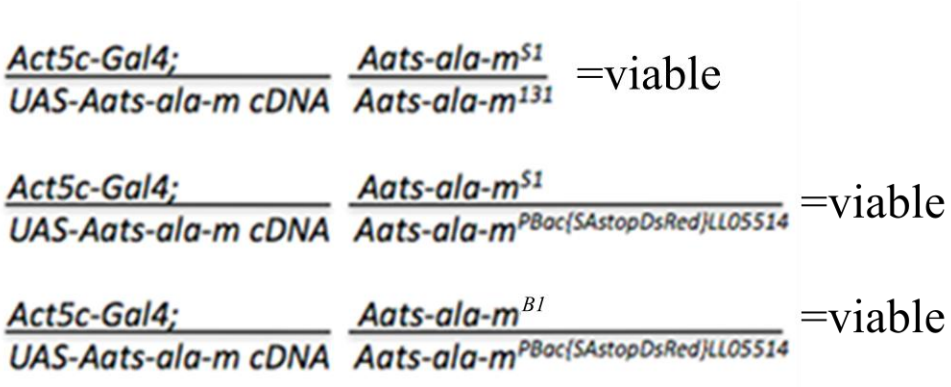

Figure 10. Expression of WT *Aats-ala-m* cDNA resuces lethality of group 6 alleles

Aats-ala-m^{S1}*/Aats-ala-m*^{B1}, *Aats-ala-m*^{S1}*/Aats-ala-m*$^{PBac\{SAstopDsRed\}LL05514}$, and *Aats-ala-m*^{B1}*/Aats-ala-m*$^{PBac\{SAstopDsRed\}LL05514}$ flies are non-viable. Expression of wild type *Aats-ala-m cDNA* rescues the lethality of these combinations of group 6 alleles.

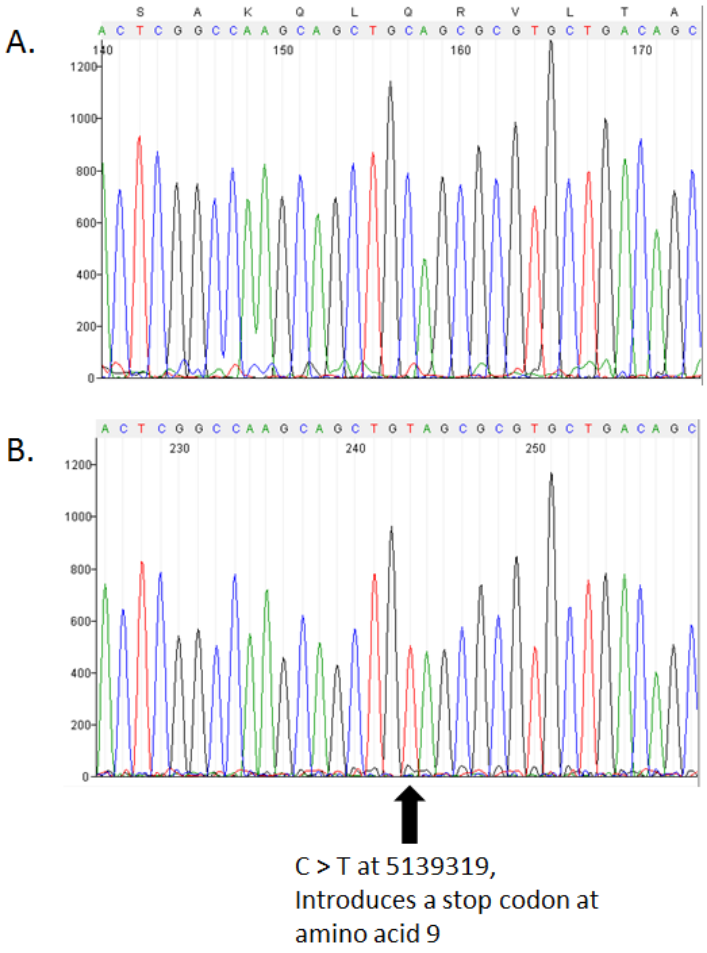

Figure 11.

The screen was performed in an isogenized background, so differences between DNA sequences between different alleles generated in the screen likely arose due to EMS mutagenesis events. A.) Sequence trace from the B1 allele from 3L: 5139302 to 5139335. This sequence is identical to the sequence from Drosophila of genotype *y; cn bw sp* from the Drosophila Genomic Sequencing Project[26].

B.) Sequence trace from the S1 allele from 3L: 5139302 to 5139335. There is a change at 5139319 that introduces a stop codon in the mitochondrial *aminoacyl tRNA synthetase gene.*

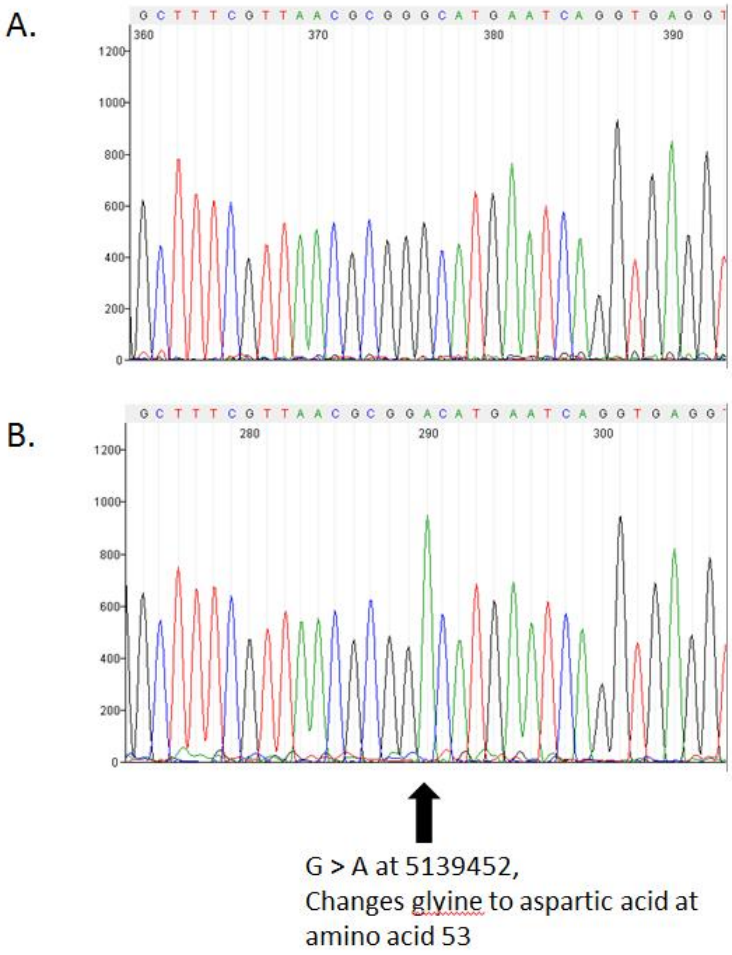

Figure 12.

A.) Sequence trace from the S1 allele from 3L: 5139436

to 5139469. This sequence is identical to the sequence from Drosophila of genotype *y; cn bw sp* from the Drosophila Genomic Sequencing Project.

B.) Sequence trace from the 131 allele from 3L: 5139436 to 5139469. There is a change at 5139452 that changes a glycine to aspartic acid.

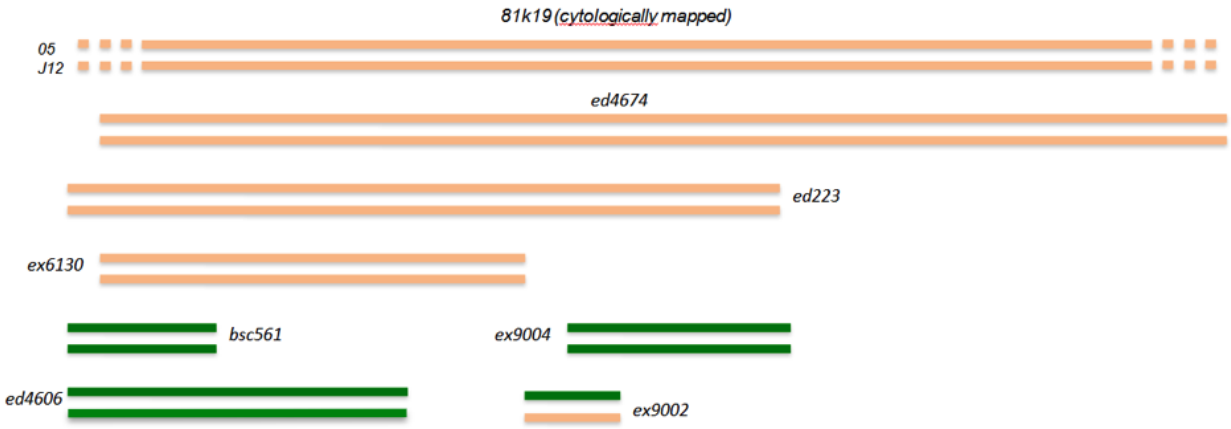

Figure 13. Group 9 deficiency mapping

Allele	Gene	result of cross between allele and bsc516	result of cross between allele and Group 1 alleles
PBac{RB}KaiRIAe01443	*KaiRIA*	non-viable	viable
PBac{SAstopDsRed}LL01229	*Leucine-rich repeat kinase*	non-viable	viable
H1	*Hairless*	non-viable	viable
bon21b	*Bonus*	non-viable	viable
P{Mae-UAS.6.11}CG5466LA00289	*cg5466*	non-viable	viable
Df(3R)PI3K92EA	*PI3K92E*	non-viable	viable
PBac{PB}Nep4c02841	*Neprilysin 4*	not tested	viable

Table 1. Complementation testing to identify the group 1 gene

Each allele likely results in reduced activity of the protein encoded by the gene, since the alleles are non-viable in combination with *bsc516*. Each allele is viable in combination with alleles from the screen, suggesting the group 1 gene is likely not one of the seven genes listed in the table. The *Neprilysin 4* allele was not tested for lethality in combination with *bsc516*, but it is likely a loss-of-function allele, since the *PBac element* is inserted in an exon of the gene and would likely disrupt the Neprilysin 4 protein.

Deficiencies tested by Juan Raudales	Deficiencies I tested
tsc677	*tsc619*
ed6085	*tsc489*
ed6096	*ed10893*
ed6220	*ed6187*
tsc461	*ex6202*
tsc140	*ex6203*
tsc495	*tsc321*
ex6210	*tsc496*
tsc749	*df(TI-F)*
tsc793	*tsc497*
ed6361	*ed6280*
	tsc874
	tsc846
	tsc547
	tsc620
	ex6214
	tsc503
	tsc504
	ex7378
	ed6346
	ed50003

Table 2. Deficiency mapping to identify the group 5 gene

Deficiencies were tested with group 5 alleles to map the group 5 gene

Deficiencies tested by Stephen Fleenor	Deficiencies tested by Juan Raudales	Deficiencies I tested
bsc289	*Aprt-1*	*bsc181*
bsc181	*pbl-x1*	*M21*
ed4287		*ZN47*
bsc119		*XD198*
ex6092		
bsc428		
bsc671		
bsc672		
ed208		
ex6098		
ed4341		
ex9058		
ed210		
bsc371		
ex6106		
ex6107		
bsc437		
ex7210		
bsc411		
ex6109		
bsc224		
bsc117		
ex8104		
bsc375		
bsc732		
bsc815		
bsc389		

Table 3. Deficiency mapping to identify the complementation group 8 gene

Chapter 3: Discussion

In this work, I identified *Aats-ala-m* as a gene that interacts with *lqf*. This result likely means that *lqf* and *Aats-ala-m* interact in a common genetic pathway. It is possible that Aats-ala-m interacts with Lqf through a Notch signaling related activity of Lqf or an activity of Lqf not related to Notch signaling. How might Aats-ala-m interact with lqf? Aats-ala-m might interact with Lqf through its role in translation in the mitochondria. Decreased mitochondrial translation in Aats-ala-m mutants might lead to decreased levels of proteins of the electron transport chain, and decreased cellular ATP and GTP. A certain cellular process related to Lqf function might be highly energy dependent, and *Aats-ala-m* and *lqf* genetically interact because *Aats-ala-m* is required to maintain proper energy levels required for this process. It is also possible that *Aats-ala-m* mutation causes a reduction in ATP levels which disrupt many cellular processes, including functions of Lqf. Alternatively Aats-ala-m might have an extra-mitochondrial function in the cell in addition to its mitochondrial activity, and Aats-ala-m interacts with Lqf through the secondary function.

Additional findings were that I identified narrow chromosomal regions containing the genes mutated in complementation groups one, two, and nine. Additional work, especially sequencing candidate genes from alleles of the complementation groups, would likely lead to the identification of the genes mutated in these groups.

Chapter 4: Materials and Methods

Fly Crosses

All fly crosses were carried out at 25° C.

Sequencing the *S1* allele

For the *S1* allele, I generated a fly stock of the genotype: *S1, lqf^{FDD9}/TM6B, GFP*. *S1, lqf^{FDD9}/TM6B, GFP* flies were put in a container with a petri-dish of solid agar-apple juice, and the flies laid eggs on the petri dish. *S1, lqf^{FDD9}* homozygous first instar larvae were collected using a microscope projecting UV light, by virtue of the fact that these larvae lack GFP expression. Larvae were collected and homogenized with a mortar and pestle in squishing buffer: 10 mM Tris-HCl pH8, 1 mM EDTA 25 mM NaCl, 200 µg/mL Proteinase K. I used 1 larvae per 10 µL squishing buffer. Once homogenized, the larvae were incubated at 37° C for 3o minutes, and 95° C for 2 minutes. I performed PCR to amplify regions of the Aats-ala-m gene from this DNA. Three PCR products were generated, using three different sets of primers, corresponding to the beginning, middle, and end parts of the Aats-ala-m gene. Once I obtained PCR product, I cloned the PCR product into the pCR-Blunt II-TOPO vector using the Invitrogen Zero Blunt TOPO PCR cloning kit. The vector containing PCR product was transformed into E. Coli, and I plated the transformed E. Coli onto LB plates containing Kanamycin. Colonies on the plates were of bacteria that were transformed with the vector-PCR product, since the

vector contains Kanamycin resistance. I then grew the transformed bacteria in liquid LB, and used a phenol-chloroform plasmid isolation protocol to isolate the vector-PCR product. I sequenced the PCR product from the vector using M13 Reverse and T7 primer sites located within the vector. For the Aats-ala-m middle and end part PCR products, the entire PCR products were sequenced, and I did not find any mutations when the sequenced DNA was compared to the Drosophila sequences by BLAST alignment, or the DNA sequences from the *131* allele (described below). For the Aats-ala-m beginning part PCR product, several separate PCR reactions were carried out, and the PCR products were individually transformed and sequenced. Each sequencing result showed the mutation at 5139319.

Sequencing the *131* allele

I was not able to obtain lqf^{FDD9} *131* homozygous larvae, so I obtained DNA from *131* homozygous flies in another way. I generated flies that were of the genotype: *Act5c-Gal4/UAS-Aats-ala-m-cDNA; Aats-ala-*m^{131}*/Aats-ala-*m^{131}. These flies live to the adult stage. I obtained DNA from adults of this genotype and carried out PCR reactions to amplify the beginning, middle, and end parts of *Aats-ala-m*. The PCR reactions should only amplify DNA from the third chromosome, and not from the *UAS-Aats-ala-m-cDNA* rescue construct, since the primers for the PCR reaction are designed towards *Aats-ala-m* intronic sequences, or sequences outside the *Aats-ala-m* gene region. The PCR

product was sequenced directly using the PCR primers. I sequenced the entire *Aats-ala-m* middle and end part PCR products and did not find a mutation in these regions. For the *Aats-ala-m* beginning part, I sequenced PCR product from two separate PCR reactions. Both sequencing results yielded the 5139452 mutation.

Generating *UAS-Aats-ala-m-cDNA* rescue construct

I obtained LD11251, which is Wild-Type *Aats-ala-m* cDNA contained within the pBluescript SK- vector. I transformed LD11251 into *E. Coli* and obtained a culture of E. Coli containing LD11251. I grew this culture of *E. Coli* and performed a mini-prep to purify LD11251. Once purified, I excised the cDNA insert from pBluescript using NotI and KpnI restriction enzymes. I restricted the pUAST vector with NotI and KpnI and ligated the insert into pUAST. I transformed pUAST-Aats-ala-m-cDNA into E. Coli and obtained cultures containing pUAST-Aats-ala-m-cDNA. I purified pUAST-Aats-ala-m-cDNA by midi-prep. I performed sequencing of pUAST-Aats-ala-m-cDNA to assure that I generated the correct vector-insert. Drosophila were transformed with pUAST-Aats-ala-m-cDNA by Genetivision (Houston, TX).

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Vita

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